A STUDY OF SYSTEMIC FUNGAL INFECTIONS IN RENAL TRANSPLANT RECIPIENTS

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DECLARATION

I solemnly declare that the dissertation titled ***"A STUDY OF SYSTEMIC FUNGAL INFECTIONS IN RENAL TRANSPLANT RECIPIENTS"*** is done by me at the Department of Nephrology, Madras Medical College & Govt. General Hospital, Chennai, during August 2008 – December 2011 under the guidance and supervision of Prof**. Dr.N.GOPALAKRISHNAN.M.D., D.M., FRCP.**

The dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University towards the partial fulfillment of requirements for the award of **D.M., degree in Nephrology.**

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CERTIFICATE

This is to certify that the Dissertation titled, **"A STUDY OF SYSTEMIC FUNGAL INFECTIONS IN RENAL TRANSPLANT RECIPIENTS"** is the bonafide record work done by *Dr.N.D.SRINIVASA PRASAD*, under our guidance and supervision in the Department of Nephrology, Government General Hospital, Madras Medical College, Chennai, submitted as partial fulfilment for the requirements of D.M. Degree examination Branch III NEPHROLOGY, AUGUST 2011, under The Dr.M.G.R. Medical University, Chennai.

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BACKGROUND:

Two major factors for successful organ transplantation are better control of rejection and better prevention and treatment of infections. In renal allograft recipients, immunosuppressive drug therapy is the major cause of immunocompromised status and occurrence of infections, which arise most commonly as a result of invasion by endogenous opportunists. It may also follow colonization by exogenous environmental organisms and via transfer of cytomegalovirus along with the transplanted kidney. The overall incidence of opportunistic infections varies from center to center; up to 15% of renal transplant recipients die of these infections. Clinical signs and symptoms of infection in immunocompromised patients may be concealed or imitated by the underlying disease, and a high index of clinical suspicion is vital. The unusual pathogens encountered in these patients demand thorough investigation. The success of management of opportunistic infections depends on strong clinical suspicion, early diagnosis, and prompt treatment. The challenges of early diagnosis of opportunistic infections and prompt treatment are great; the rewards are even greater.

Fungi are one of the important causes of opportunistic infection in renal transplant recipients. Invasive fungal infections are among the most important causes of mortality among transplant patients. The incidence of these infections is increasing due to greater number of transplant surgeries and usage of potent immunosuppression. Although fungal infections are less common among kidney transplant recipients (1- 14%), they are responsible for significant mortality in this group of patients. The occurrence of invasive fungal infections is highest in the early post transplant period, when immunosuppression is greatest. Prolonged antifungal therapy and surgical intervention are needed for control of fungal infections. Early and prompt diagnosis of the condition and intervention is required to prevent morbidity and mortality.

AIM

To study the clinical profile, risk factors for acquiring fungal infections, its outcome and the factors influencing outcome in living and deceased donor renal transplant recipients.

REVIEW OF LITERATURE

INTRODUCTION:

Despite technical, immunological, and therapeutic advances in the field of renal transplantation, infections remain a major barrier to a successful outcome. The high mortality associated with infections emphasizes the negative impact of immunosuppression on the recipient's immune system. More than 80% of renal transplant recipients suffer at least one episode of infection within 1 year of transplantation.[3]

Fungal infections after solid organ transplantation, despite a lower incidence than bacterial and viral infections, remain a major cause of morbidity and mortality. Fungal infections in different types of solid organ transplantation show various incidences, underlying pathogenesis, and modes of clinical presentation.[2] As many as 14% of renal allograft recipients, 32% of heart recipients, 35% of heart-lung, 38% of pancreas recipients, and 42% of liver recipients have been reported to develop clinically significant fungal infections.[4] Among fungi, the responsible pathogens include Cryptococcus neoformans, Aspergillus species, Candida species, Coccidioidomyces immitis, Histoplasma capsulatum, and Mucormycosis.

WHAT MAKES FUNGAL INFECTIONS SPECIAL?

Fungi are eukaryotic organisms like humans. They have a more complicated genome and they synthesize proteins in a complex manner. Their replication process is also complex. And also fewer medications are available for their treatment. Even though they are less invasive, they are ubiquitous. Because of their complex structure, their eradication is difficult requiring long duration of medical therapy and surgical debridement. There is no vaccine available to prevent fungal infection.[6]

The humans are protected from fungal invasion by Th1 helper cell response and macrophage activation. The human immune system mounts a type IV reaction against the fungal pathogen. The human body is not protected by Ab response. And antibody titre is not useful in diagnosis. But antigen detection is useful in diagnosis.

RISK FACTORS FOR THE DEVELOPMENT OF FUNGAL INFECTION[6]

- Anti Thymocyte Globulin (ATG) usage
- Multiple anti rejection therapies
- Neutropenia, antibiotics

- Cytomegalo virus (CMV), Hepatitis C Virus (HCV) and Epstein Bar Virus (EBV) infections
- Graft dysfunction (serum creatinine > 2 mg/dl)

Invasive fungal infection has a high mortality rate, because the infection is often advanced at the time of diagnosis and the disease is rapidly progressive. There is difficulty in establishing early diagnosis. The efficacy of therapeutic agents is limited by toxicity and drug interactions.

TIME TABLE OF INFECTIONS IN RENAL TRANSPLANT RECIPIENTS[3]

0–1 MONTH

Bacterial: wound infection, pneumonia, urinary tract infection, pyelitis, bacteremia

Viral: herpes simplex, hepatitis

1–6 MONTHS

Viral: CMV, Epstein–Barr, varicella zoster

Fungal: Candida, Aspergillus and Cryptococcus

Bacterial: Listeria, Legionella, Nocardia

Protozoa: Pneumocystis carinii

> 6 MONTHS

Bacterial: community micro-organism, mycobacteria

Viral: hepatitis B or C, CMV chorioretinitis

FUNGAL PATHOGENS ASSOCIATED WITH INFECTION[4]

PREDOMINANT FUNGI

Candida spp

Aspergillus spp

Cryptococcus neoformans

EMERGING FUNGI

Fusarium spp

Trichosporon spp

Malasezzia furfur

Scedosporium spp

Zygomycetes

Dematiaceous moulds

ENDEMIC FUNGI

Histoplasma capsulatum

Blastomyces dermatitidis

Coccidioides immitis

SYSTEMIC FUNGAL INFECTIONS: COMPARATIVE DATA[1]

	Gallis et al (n-171)	Nampoory et al (n-512)	John et al (n-920)	Jayakumar et al (n-362)	PGI-CHD (n-850)
Fungal infection	13%	3.7%	5.6%	19%	9.8%
Candidiasis	2.3%	1.6%	1.4%	13.8%	2.8%
Cryptococcosis	5.8%	0.5%	2.4%	0.8%	1.9%
Aspergillosis	1.2%	0.9%	1 %	3 %	2.3%
Mucormycosis	1.2%	0.4%	1.1%	1.5%	2.0%
Others	0.6%	-	0.9%	-	0.5%

INCIDENCE OF INVASIVE FUNGAL INFECTIONS[2]

TRANSPLANT TYPE	INCIDENCE OF INFECTIONS (%)
Renal	1.4 - 14
Heart	5.0 - 21
Liver	7.0 - 42
Lung & Heart/Lung	15.0 - 35
Pancreas	18.0 - 38

PGI CHANDIGARH EXPERIENCE - 5 YEARS: [9]

Numbers of transplant follow up patients	245
Invasive fungal infection	15
Rhino cerebral Mucormycosis	3(20.00%)
Pulmonary Mucormycosis	2(13.34%)
Candida Septicemia	2(13.34%)
Pulm. Aspergillus	2 (13.34%)
Aspergillus wound infection	2(13.34%)
Aspergillus wound infection with graft invasion	2 (13.34%)
Ectopic Aspergillus abscess	1 (6.67%)
Disseminated Cryptococcus	1 (6.67%)
Pulmonary involvement	4(26.66%)
Prevalence	6.12%

MORTALITY RATES IN INVASIVE FUNGAL INFECTIONS[5]

TRANSPLANT	PATHOGEN	MORTALITY (%)
Kidney	Candida spp Aspergillus spp Cryptococcus neoformans	23 – 71 20 – 100 0 - 6
Liver	Candida spp Aspergillus spp Cryptococcus neoformans	6 – 77 50 - 100 0 – 22
Lung or Heart/Lung	Candida spp Aspergillus spp	27 21 – 100
Heart	Aspergillus spp	32 – 64
Pancreas	Candida spp	20 – 27

AN OVERVIEW OF ANTIFUNGAL AGENTS

AMPHOTERICIN B

Amphotericin B is the traditional drug of choice for most of the fungal infections. It is a rapidly effective fungicidal drug. It binds to ergosterol in fungal membrane and punches holes in the membrane leading to leakage of potassium and other intracellular molecules. It stimulates innate immune cells & also interacts with adaptive humoral immunity. It also increases levels of antibodies against fungal HSP90.[6]

Acute infusion related toxicity correlates with increase in level of inflammatory cytokines. The most prominent disadvantage of amphotericin B is its nephrotoxic effect.[8] The treatment of aspergillosis with amphotericin B in solid organ transplant recipients results in a higher incidence of nephrotoxicity because of the concomitant use of cyclosporine.[49] Liposomal amphotericin B (AmBisome) has far fewer side-effects and can be much more safely used in patients with solid organ transplants, despite concomitant use of cyclosporine A. In a series of 187 transplant recipients, liposomal amphotericin B was discontinued due to side-effects in 3% of cases. The overall mean increase in serum creatinine levels was 20%. Other side-effects included low serum potassium concentrations (36%) and a rise in alkaline phosphatase levels (26%).[50]

Liposomal amphotericin B markedly decreases the death rate due to aspergillosis in neutropenic patients after bone marrow transplantation from 90% down to 25% even when neutrophil counts are still low.[50] The antifungal efficacy of AmBisome seems to be related to its ability to target fungi rather specifically.[51]

AZOLES:

Among azoles, fluconazole has been used effectively in localized Candida infections, and may also be an option in the treatment of systemic candidiasis,[52, 53] but data supporting its use in pneumonia are lacking at the present time. Itraconazole or the new azole voriconazole[55] are effective therapies against aspergillosis, candidiasis or cryptococcosis. They are well tolerated. Enzyme-inducing drugs such as rifampicin and phenytoin significantly reduce the oral bioavailability of Itraconazole, and plasma monitoring of its plasma concentration is recommended when enzyme-inducing agents are co administered.[54]

Itraconazole has been shown to be as effective as amphotericin B in small series of neutropenic patients,[56, 55] and in liver or heart transplant recipients.[56, 57] Failure of Itraconazole treatment of pulmonary aspergillosis in heart transplant recipients has been reported, but most of these patients had been maintained on high-dose steroids.[59, 60]

ECHINOCANDIN:

Caspofungin, Andidulafungin, Micafungin are all excellent fungicidals for Candidiasis and Candidemia including C. parapsilosis, krusei which may be resistant to azoles. Candins too have immuno-stimulatory

properties.[61] They enhance fungal killing by neutrophils and macrophages which is done by unmasking β glucan.

Only IV formulations are available. No dose change is required for renal impairment. Caspofungin is approved for salvage treatment of invasive aspergillosis. It has got low toxicity profile which may include some LFT abnormalities. Secondary resistance is associated with point mutations in the Fks1 gene of β-D-glucan.[62] There are recent reports of secondary resistance against candida and aspergillus species.

Modulation of immunosuppression has to be taken into account as a major component of fungal infection treatment. The use of hematological growth factors may prove to be useful in the near future in not only restoring the neutrophil count more quickly, but also increasing the capacity to contain fungal pathogens.[60]

INDIVIDUAL FUNGAL INFECTIONS

ASPERGILLUS INFECTIONS IN SOLID-ORGAN TRANSPLANT RECIPIENTS

Lungs and sinuses are primary site of infection. Use of catheters may cause primary cutaneous infection. Dissemination to CNS and other organs is common. High mortality occurs especially in lung and liver recipients.[10]

PULMONARY INFECTION:

Airborne conidia (2.5-3 microns) are infective. Symptoms include fever, cough, dyspnea, pleuritic pain and hemoptysis, Necrotizing bronchopneumonia, single or multiple abscesses. Early diagnosis difficult. Radiographs often normal. Culture often negative. "Halo" sign on chest CT scan highly suggestive of Aspergillus. Definitive diagnosis is made from biopsy of lung tissue.[11]

BLOOD VESSEL INVASION:

Invasion of blood vessel causes thrombosis, tissue infarction, hemorrhage and hematogenous dissemination.[13]

CNS INFECTION:

Presents either as manifestation of disseminated infection or as isolated CNS disease. Non-specific symptoms include fever, subtle change in level of consciousness. Also presents as abscess formation or less commonly meningitis.MRI lesions found at grey/white matter junction. Definitive diagnosis is by brain biopsy if no extraneural site more accessible.[14]

INVASIVE PULMONARY ASPERGILLOSIS:

Classically, the major risk factors for invasive pulmonary aspergillosis include severe or prolonged neutropenia (absolute neutrophil count $<500\times10^{6}\cdot L^{-1}$) and prolonged high-dose corticosteroid therapy. [21] In the

absence of an effective host immune response, the spores mature into hyphae that can invade the pulmonary structures, particularly blood vessels. This results in pulmonary arterial thrombosis, hemorrhage, lung necrosis and systemic dissemination.[18]

Macrophages and granulocytes are the major immunoregulatory cells involved in host defenses against fungal infections. It has been demonstrated that corticosteroids suppress macrophage and granulocyte function, whereas little effect of the suppression of T-lymphocyte function by cyclosporine has been noted. [22]

The isolation of Aspergillus from bronchoalveolar lavage fluid and/or sputum has been shown to correlate with the histopathological changes of invasive pulmonary aspergillosis in bone marrow recipients[23] in whom invasive forms cause the highest mortality.[19] Aspergillus isolation from culture of respiratory secretions, pleural fluid or ascitic fluid has also been correlated with invasive Aspergillus infection and poor outcome in recipients of both liver and kidney transplants.[24] In cases in which the diagnosis need to be proven, transbronchial biopsy is usually of little sensitivity, being as low as 20%, [26] whereas transthoracic needle biopsy or open lung biopsy provides a higher and more specific diagnostic yield.[27]

Mortality rates from infections can be high (50–70%) and patient outcome depends on the early institution of antifungal therapy, the severity of the underlying disease and the speed of granulocyte recovery.[21, 28]

Invasive aspergillosis has been described as occurring following up to 18% of heart and lung transplants, [29] but mortality can be reduced with pre-emptive therapy and reduced immunosuppression.[12, 30]

Invasive pulmonary aspergillosis appears on radiographs as multiple ill-defined 1–2-cm nodules that gradually coalesce into larger masses or areas of consolidation.[31] An early computed tomography finding, but seen with thin collimation, is the rim of ground-glass opacity surrounding the nodules (computed tomography halo sign).[31] This sign is, however, nonspecific and has also been described in patients with tuberculosis, mucormycosis and Wegener's granulomatosis.[7] Cavitation is usually a late finding. The intracavitary mass composed of sloughed lung and the surrounding rim of air may be seen as “the air crescent sign”. Lobar consolidation is more common and less specific.[33] Pleural effusion is unusual and adenopathy rare.

OTHER FORMS OF DISEASE RELATED TO ASPERGILLUS

Chronic necrotizing or semi-invasive aspergillosis typically occurs in patients with mild immunosuppression such as occurs in chronic obstructive pulmonary disease, sarcoidosis or underlying malignancy. It progresses slowly over a period of weeks or months. Aspergilli invade the tissues adjacent to cavities, increasing their size due to progressive necrosis. In transplantation, these slowly invasive forms are not described as distinct entities compared with the acute invasive forms.[25]

Allergic bronchopulmonary aspergillosis has occasionally been described after transplantation.[20] Milder forms may have local consequences such as bronchocentric granulomatosis that may be underestimated. The physiopathology of allergic bronchopulmonary aspergillosis and bronchocentric granulomatosis may be better understood in the near future with the recent observation that aspergilli share epitopes similar to the cytoplasmic structures of epithelial cells. These local infections could trigger autoimmune processes.[36] It is possible that such phenomenona do occur in the lungs of transplant recipients, leading to bronchial inflammation and stenosis.

Aspergilloma can be present in pre-existing pulmonary cavities before transplantation. With immunosuppression, aspergilli can invade adjacent structures and lead to widespread disease.[29] Preventive surgical removal of such mycetoma remains a matter of debate, especially for mycetoma resulting from previous invasive aspergillosis after bone marrow transplantation.[37]

DIAGNOSIS: [17]

1. Radiology: chest X-ray and CT: halo sign

2. Microbiology Respiratory secretions: BAL/biopsy - Direct microscopy and Culture.

3. Serological surveillance: ELISA for galactomannan and Beta D glucan assay.

4. Polymerase Chain Reaction.

GALACTOMANNAN TEST - ASPERGILLUS ANTIGEN DETECTION[16]

It is an Immunoenzymatic sandwich enzyme immunosorbent assay (EIA). It uses a monoclonal antibody to GM polysaccharide antigen in fungal cell wall. Test duration is 3 hours. Specimen tested includes serum and tissue/fluid obtained from Broncho-alveolar lavage. Recommendation refers true positive only when more than one sample is positive. Positive

predictive value of the test is 71% and Negative predictive value is 88%. It has a Sensitivity of 50-94% and Specificity of 81-99%. False positive can occur with other fungi, translocation of gm antigen from food through damaged intestinal mucosa (e.g. Bread, cereal, rice, turkey) and mould-derived antibiotics e.g. Penicillin. Use of β-D-glucan + GM ELISA in combination increases specificity & PPV.

ASPERGILLUS PCR ASSAYS

Detects 1-10 cfu/ml -Blood, BAL and Tissue not standardized. No commercial assays available. It has got variable sensitivity & specificity. For 'in-house' assays it has a sensitivity of 79-100% and specificity of 81-100%.[56] Also antifungal treatment clears DNA from blood.

BETA D GLUCAN ASSAY

It is a pan fungal assay. It has a good negative predictive value 100 %. The test is positive in aspergillus, candida, fusarium, trichosporan, sachharomyces, and acremonium. Interestingly it is negative in Mucor. [55]

TREATMENT OF INVASIVE ASPERGILLUS INFECTION

Voriconazole is drug of choice. Liposomal amphotericin is second choice. Fluconazole is not useful. Itraconazole can be tried if disease is not very invasive. Posaconazole has also been reported to be efficacious.

Echinocandins are also useful. Surgical debridement is the final resort and had to be considered in appropriate cases.[41]

VORICONAZOLE

It is given in a dose of 6 mg / kg IV for one day, followed by 4 mg / kg IV bd. Oral bioavailability is > 95 %. Oral dose is 400 mg bd, and then 300 mg bd. Side effects include photopsia and LFT changes. In renal failure single dose IV is given followed by oral, because intravenous form has cyclodextrin which is retained in renal failure. Voriconazole has got several drug interactions. It is contraindicated with amphotericin. Rifampicin, eptoin and barbiturates reduce levels of voriconazole. QT prolongation can occur, hence drugs like cisapride etc., has to be avoided. Tacrolimus has to be reduced by one third and Cyclosporine has to be reduced by 50 %. Levels have to be monitored and dose has to be adjusted again after stopping voriconazole. [42]

AMPHOTERICIN

Amphotericin B is given in a dose of 1 to 1.5 mg/kg/day. Liposomal amphotericin B can be safely used up to a dose of 3 mg/kg/day. An intravitreal injection of amphotericin B (10mg) should be given for

endophthalmitis. Conventional amphotericin B should be avoided in renal failure.[43]

ASPERGILLUS PREVENTION: [40]

Reduction of environmental risk factors

– Gardening, Wood chips/mulch

– Horse manure

– Construction sites.

CANDIDA INFECTIONS IN SOLID-ORGAN TRANSPLANT RECIPIENTS[47]

It is the most common infection ranging from trivial to fatal. Serious infections are increasing. Highest risk of infection is in liver and pancreas recipients. Type and site of infection depends on species colonizing oropharynx and GI tract, use of invasive techniques and presence of drains and catheters.

Indwelling devices may result in Cystitis and retrograde infection of pancreatic graft / renal graft from bladder catheters can occur. Vaginal colonization also contributes to spread of infection. Colonization of GI Tract may result in hematogenous spread due to disruption of intestinal mucosa. Contamination of peritoneal cavity results in peritonitis. Biliary obstruction,

hepatic and splenic abscesses has also been reported. Hematogenous spread does occur from central venous catheters.

CHANGING PROFILE OF CANDIDA INFECTION

Data from Sir Gangaram Hospital showed that Non albicans candidiasis constituted about 72%.

Candida haemuloniiis an emerging pathogen constitutes about 10% of the non albicans species. This pathogen is resistant to Amphotericin B. Minimum Inhibitory Concentration of C lusitaniae, C guilliermondii and C parapsilosis was very high to Amphotericin B.[57]

Prevalence Data from Safdurjung Hospital (Sept 2003 –Nov 2004) - Of total 362 samples including 152 from blood, Candida was isolated in 102 samples. 75.4% of the overall isolates were Candida non albicans. In Blood and other sterile sites Candida Non albicans were isolated in ~90% cases. Incidences of Amphotericin B resistance were as high as ~7%. This high resistance is attributable to a reduction in Membrane ergosterolin resistant mutants of some Candida spp.[58]

Reports from Sanjay Gandhi Post-Graduate Institute of Medical Sciences revealed infection due to non-Candida albicans species is more

common than C. albicans. The incidence of Candida Non Albicans was 67%. The mortality with Candidemia patients was 55% (SLIDE 6).[56]

Reports from AIIMS Hospital in a 5 Year study (2001 -2005) revealed that the incidence of Non-albicans Candida was 79 -80%. C.tropicalis was the most commonly isolated pathogen –35.3%. High incidence of C. glabrata with 17.5% was seen. Anti-fungal resistance was found in 11.7%. Mortality in the first 4 years was high –72.2%, which was probably because of unawareness of disease prevalence.

INVASIVE CANDIDA SPECIES IN SOLID ORGAN TRANSPLANTS: [48]

C. albicans (46%)

C. glabrata (25%)

C. krusei (3%)

C. tropicalis (4%)

C. parapsilosis (8%)

C. Lusitania (1%), Multiple (11%), Other (2%).

TREATMENT OF CANDIDA INFECTIONS

Indwelling venous catheters has to be removed. Eye examination has to be done to rule out endophthalmitis. Cultures and speciation has to be obtained as non-albicans strains are increasing.. Fluconazole is useful for C.albicans and all other species except C. glabrata or krusei. C. glabrata is

becoming increasingly resistant to Fluconazole. Fluconazole has to be used with caution in critically ill patients. When Candidemia or invasive infection is present Caspofungin, Micafungin, and Anidulafungin are initially used until species available. These drugs should not be used with cyclosporine. Amphotericin B 0.5 - 0.7 mg/kg/day or Lipid formulation of Amphotericin B 3 mg/kg/day is reserved for refractory disease.[39]

CRYPTOCOCCOSIS

Cryptococcus (yeast) is causing human disease particularly in immunosuppressed. It has a worldwide distribution. 20% of non-HIV-associated Cryptococcus infection occurs in solid organ transplant recipients. Infection is acquired through Pigeon droppings, soil, decaying wood chips etc.[32]

PULMONARY INFECTION:

Primary infection occurs via inhalational route. Pulmonary infection may be asymptomatic (e.g., nodule) or it can also present as pneumonia. Chest radiograph shows nodules, infected lymph nodes, masses and consolidation. Cavitation, pleural effusion and adenopathy are less common.[32]

CNS INFECTION:

Hematogenous dissemination to CNS can occur resulting in meningitis and Cryptococcoma. Increased intracranial pressure (>200 mm H2O) is common and usually fatal. Symptoms include headache, fever, visual disturbances, nausea, vomiting, and cranial nerve abnormalities.[34] May have subtle symptoms for months like altered personality, wide unsteady gait, low grade fevers. An India-ink smear of centrifuged spinal fluid sediment may reveal encapsulated yeasts in at least half of patients. Capsular antigen may be detected in cerebrospinal fluid by latex agglutination.CSF polysaccharide antigen is very reliable for diagnosis. Serum antigen test may be negative. MRI can rarely show Cryptococcoma.[35]

TREATMENT

PULMONARY INFECTION:

200 - 400 mg/day fluconazole or Itraconazole (200mg BID) for 6 to 12 months

Severe infection: Amphotericin B 0.7 mg/kg/day ± 5-FC (100mg/kg/day) initially, then switch to oral fluconazole, 400 mg/day after clinically improved and continue for 6 to 12 months.

Surgical resection may be needed for individuals with extensive lobar consolidation or large mass lesions.[34]

CNS INFECTION:

Amphotericin B or lipid formulation + 5-FC for at least 2 weeks until CSF culture negative--need LP at 2 weeks.

Consolidation of lung - with oral fluconazole 400 mg/day for 10 weeks, then 200 mg/day for 6 to 12 months. Shunting or frequent LP's may be needed for increased ICP. Large CNS lesions may require surgery.[35]

PREVENTION:

Treat pretransplant cryptococcal infection aggressively.

Screen high-risk donors, e.g. gardeners, pigeon breeders.

MUCORMYCOSIS**:**

It can be easily differentiated from Aspergillus by the following features. Mucor has an Aseptate hyphae, it branches at 90 degree angle and it is thicker (10 to 20 um).[38]

CLINICAL PRESENTATION:

Rhino cerebral involvement is the most common. It especially affects diabetics, though it can affect any immunosuppressed individual. Patient presents with fever, unilateral facial pain, nasal congestion, epistaxis, visual disturbance, proptosis, periorbital cellulitis, Cranial nerves II, III, IV, VI – ophthalmoplegia and black necrotic lesion in hard palate and nose.[39]

Patient can also present with pulmonary, cutaneous or gastro-intestinal involvement. Though disseminated mucormycosis is less common, it can also occur.

Complications include Cranial Nerve dysfunction, Cavernous sinus and internal carotid artery thrombosis, cerebral abscess. Differential diagnosis includes Aspergillus, bacterial sinusitis and periorbital cellulitis.[38]

TREATMENT

Surgical debridement is the most useful treatment modality and has to be resorted to it as early as possible.

Medical management consists of Amphotericin B and caspofungin. Triazoles are not useful. Voriconazole treatment may predispose to mucormycosis. Echinocandins are not useful as a single agent. It has to be combined with Amphotericin B.

PNEUMOCYSTIS JEROVECII

This is a frequent complication in transplant patients who do not receive prophylaxis with trimethoprim – sulfamethoxazole. The risk of PJP is increased in patients who have developed steroid-resistant rejection, CMV

infection, or other immunomodulating infections such as tuberculosis and hepatitis C.[56]

Transmission is usually person to person and air borne. The patient with *P*. jerovecii pneumonia usually presents with fever and dyspnea. Physical signs are often absent on examination. Some patients may show eosinophilia. Severe hypoxemia is usually present. Interstitial pneumonia is frequent, but X-radiographic abnormalities may be variable and not specific.

DIAGNOSIS:

Chest X-Ray shows diffuse bilateral interstitial infiltrate or few infiltrates. CT scan shows ground glass opacities or cystic lesion (SLIDE 7). A correct diagnosis can be made only by BAL. Methanamine silver or toludine blue or cresyl violet and calcofluor are the stains that can be used. Direct FAT and PCR is available in few centers. Beta D glucan assay is also useful.

TREATMENT:

High-dose trimethoprim–sulfamethoxazole is the treatment of choice. The recommended dose is 15 mg/kg of trimethoprim, divided into 3–4 doses. A treatment of 14 days is usually sufficient. In patients allergic to

sulfonamides, slow intravenous infusion of pentamidine, at doses ranging between 3 and 4 mg/kg per day according to the severity of the disease, may be indicated. Injection Pentamidine is nephrotoxic and also causes hypoglycemia, hypotension and pancreatitis. Alternative treatment includes clindamycin, 900 mg every 8 hours, plus primaquine, 15 mg by mouth, every day.

X-RADIOLOGIC FINDINGS IN POST-TRANSPLANT PNEUMONIA

Radiographic abnormality	Acute development	Chronic development
Nodular infiltrate	Bacteria	Fungi, Nocardia, tuberculosis, pneumocystis jerovecii
Cavitation	Bacteria (Legionella), fungi	Tuberculosis
Peri bronchovascular abnormality	Bacteria, viruses (influenza)	Fungi, CMV, Nocardia, tuberculosis, pneumocystis jerovecii
Consolidation	Bacteria (Legionella)	Fungi, viruses, Nocardia, tuberculosis, pneumocystis jerovecii
Diffuse interstitial infiltrates		Fungi, CMV, pneumocystis jerovecii

COCCIDIOIDOMYCOSIS

Coccidioides immitis is a soil saprophyte. Infection results from the inhalation of wind-borne spores arising from soil sites. In renal transplant recipients the infection may occur in endemic areas, and can present in the form of pneumonia or disseminated disease.[44]

Pneumonia presents with non-specific symptoms such as fever, malaise, dry cough, headache, and dyspnea. Eosinophilia and erythematous skin lesions may be found in a few patients. X-radiologic findings show segmental pneumonitis, mild infiltrates, hilar adenopathy, and pleural effusion. Cavitation and solitary nodules (coccidioidoma) may develop in asymptomatic patients. The disseminated form is characterized by fulminant respiratory failure, disseminated intravascular coagulation and profound hypotension mimicking bacterial pneumonia and septic shock.

TREATMENT:

Caspofungin, amphotericin B, and azoles are usually effective.[71]

RISING OPPORTUNISTIC FUNGAL INFECTIONS

T. glabrata is a yeast-like organism, normally present commensally in the human vagina. T. glabrata pneumonias have been reported in myelosuppressed patients with neoplastic disease. T. glabrata was isolated

from the bronchoalveolar lavage fluid of three of 26 of the present authors' lung transplant patients. Pneumonia can occur and progress despite amphotericin B treatment, but apparent lung infection has also been seen to regress without specific treatment, with bone marrow recovery after bone marrow transplantation.[44]

Invasive fungal infections caused by unfamiliar species are increasingly being reported in immunocompromised patients.[45] These emerging opportunistic fungi include Fusarium, a common plant pathogen; Penicillium marneffei; Trichosporon beigelii; Blastoschizomyces capitatus and Malassezia furfur. Thus Fusarium can cause disseminated infection similar to aspergillosis in profoundly neutropenic patients.[46]

Invasive fungal infections pose a great challenge to transplant physicians, due to the lack of reliable diagnostic tests and limited therapeutic options. Invasive fungal infections are associated with a high overall mortality. Prompt diagnosis and treatment are necessary to avoid the life threatening complications and may greatly improve prognosis. .

MATERIALS & METHODS

Renal transplant recipients both cadaveric and living-related during the time period between august 2008 and May 2011 admitted with systemic fungal infections in nephrology ward were included in the study.

Detailed history, duration of post transplant status, nature of immunosuppression, duration and type of symptoms, and history of other co morbid illnesses predisposing to fungal infections like Diabetes Mellitus and viral infections like HIV, HCV and CMV were taken. Data gathered included age, sex, date of transplantation, date of diagnosis, fungal pathogen, organs affected by infection, treatment and patient outcome.

General examination and systemic examination followed by detailed examination of systems involved like eye, ENT, respiratory tract, GI tract etc. were done.

Routine investigations like urinalysis & culture, complete hemogram, blood sugar, renal function tests, liver function tests, blood culture (bacterial & fungal), imaging of brain, Para nasal sinuses, thorax and abdomen (like x-ray, USG, CT scan) were done.

Invasive investigations for tissue diagnosis and cultures like UGI scopy, bronchoscopy, nasal endoscopy and cystoscopy and tissue biopsy were done after obtaining written informed consent from the patient.

Diagnosis was made by radiological findings, positive blood or bronchoalveolar lavage (BAL) cultures and tissue biopsies. For suspected cases of pulmonary involvement, fibreoptic bronchoscopy with bronchoalveolar lavage and transbronchial biopsy was performed. Materials from transbronchial biopsy were embedded in paraffin blocks, and sections of 5mm stained with hematoxylin-eosin. BAL fluids were cytocentrifuged and stained with Papanicolaou stain and Gomori methenamine silver stain. BAL fluids were also sent for bacterial, fungal, viral and mycobacterial cultures.

Specimens for fungal isolation are plated on inhibitory mold media, brain-heart infusion agar, and mycobiotic agar (Gibco Diagnostics, Madison, Wisc.). Some fungi (Histoplasma capsulatum) can take up to 25 days to grow. Fungal cultures were incubated at 30°C for atleast 4 weeks. Plates were evaluated daily for the first 7 days and at least twice per week thereafter.

Diagnosis of invasive fungal infections was made in the presence of at least one of the following criteria: 1) histopathological evidence of tissue

invasion on biopsy specimen; 2) positive culture from deep tissue specimen such as blood, cerebrospinal fluid (CSF), peritoneal fluid; 3) KOH mount of specimen showing pseudohyphae and/or budding yeast. For diagnosis of Cryptococcus, India ink preparation of the sample (CSF) was done.

In this study esophageal candidiasis was included as systemic fungal infection. It was diagnosed by upper GI scopy and histopathological examination of mucosal biopsy.

All the patients were treated with intravenous Amphotericin B with a maximum cumulative dose of 1.5 to 2 gms. Those who had sinusitis due to mucormycosis underwent sinus surgery. Esophageal candidiasis was also treated with intravenous Amphotericin B till a cumulative dose of 500mg followed by repeat upper GI scopy. If esophageal candidiasis was persistent, another 500mg of intravenous Amphotericin B was given.

In the above patients, etiology, clinical profile, risk factors, prognostic indicators and outcome were analyzed with appropriate statistical analysis.

STATISTICAL METHODS

Microsoft excel 2007 was used to calculate mean.

Binomial test was used to analyze factors predisposing the occurrence of fungal infections.

Student t test was used for analyzing the factors influencing patient outcome.

Differences were considered to be significant if the p-value was less than 0.05.

RESULTS

This study was conducted between Aug' 08 and April' 11, in the department of nephrology, government general hospital, chennai. Twenty two patients were diagnosed with systemic fungal infections during this period. The mean age of the study patients was 35.55 yrs. The male to female ratio was 1.75:1.

The mean duration of disease before renal transplant for these patients was 16.5 yrs. And the mean dialysis duration was 7.8 yrs.

TABLE 1: PRETRANSPLANT COMORBIDITIES IN PATIENTS WITH FUNGAL INFECTIONS

Co morbidity	Number	Percentage
Diabetes mellitus	1	4.5
Systemic Lupus Erythematosus (SLE)	1	4.5
Liver disease	0	0
Human Immunodeficiency Virus (HIV)	0	0
Hepatitis B Virus (HBV)	1	4.5
Hepatitis C Virus (HCV)	1	4.5
Cytomegalo Virus (CMV)	0	0
Pulmonary Tuberculosis (TB)	2	9
Fungal infection	2	9
No comorbidities	14	64
Total	22	100

No significant comorbidity was observed in 64% of the study population.

TABLE 2: TYPE OF RENAL DONATION IN THE STUDY POPULATION

Type of transplant	Living donor	Deceased donor	Total
Number of infections	17	5	22
Percentage	77.5	22.5	100

77.5% of infections were noticed in living donor renal transplant recipients compared to deceased donor renal transplant recipients (22.5%).

TABLE 3: TYPE OF IMMUNOSUPPRESSION AND NUMBER OF FUNGAL INFECTIONS

Type of immunosuppression	CsA+AZA+PDN	CsA+MMF+PDN	TAC+MMF+PDN	Total
Number of infections	8	5	9	22
Percentage	36	23	41	100

Among the twenty two patients with fungal infections, 41% received tacrolimus (TAC), mycophenolate (MMF) and Prednisolone (PDN). Thirty six percent received cyclosporine (CsA), azathioprine (AZA) and Prednisolone. The rest received cyclosporine, mycophenolate and Prednisolone.

TABLE 4: TIME OF ONSET OF GRAFT DYSFUNCTION (GDF) AND NUMBER OF INFECTIONS

Time of onset of GDF	0 – 3 m	4 – 12 m	13 – 24 m	No GDF	Total
Number of infections	12	4	4	2	22
Percentage	55	18	18	9	100

The average time to onset of GDF was 7.1 months. Fifty five percent of patients with fungal infections developed GDF with in 3 months. Seventy three percent of infections occurred within a year.

TABLE 5: TIME OF PRESENTATION OF FUNGAL INFECTIONS AFTER TRANSPLANTATION

Time of presentation	0 – 6 months	7 – 12 months	> 13 months
Number of infections	12	2	8
Percentage	55	9	36
Mean	12		
Range	0.5 – 83 months		

Fifty five percent of patients developed fungal infections within 6 months. The mean time of presentation is 12 months (range 0.5 – 83 months).

TABLE 6: FACTORS PREDISPOSING THE OCCURRENCE OF FUNGAL INFECTIONS

Factors	Present	Absent	Percentage	P-value
GDF	20	2	91	0.000
Surgical procedure	3	19	14	0.001
Post Transplant DM	6	16	27	0.052
HBV	2	20	9	0.000
HCV	3	19	14	0.001
CMV	11	11	50	1.000
Bacterial infections	12	10	55	0.832
Anti Rejection Therapy	8	14	36	0.286
Leucopenia	11	11	50	1.000
Anemia (<11g/dl)	9	13	41	0.523
Thrombocytopenia	9	13	41	0.523

Binomial test has been used for analyzing the above table (p-value < 0.05 is significant)

Graft dysfunction alone seemed to be a risk factor for the occurrence of fungal infection. Though many patients received anti rejection therapy (ART, 36%) and cytomegalovirus (CMV, 50%) and bacterial infections (55%), leucopenia (55%), anemia (41%) and thrombocytopenia (41%) were present in many patients, they did not predispose to the occurrence of fungal infections. (No statistical significance).

TABLE 7: FUNGAL INFECTIONS AND SITE OF OCCURRENCE

Site of fungal infection	Number	Percentage
Gastro intestinal tract	6	22
Lung	6	22
Upper respiratory tract	4	15
Urinary tract	6	22
Blood stream	3	11
Central nervous system	2	8

Fungal infections commonly occurred in gastrointestinal tract (GIT), lung and urinary tract, each 22%. Other sites were upper respiratory tract (15%), blood stream (11%) and central nervous system (CNS, 8%).

TABLE 8: TYPES OF RENAL HISTOPATHOLOGY

Histopathology	Number	Percentage
Acute cellular rejection	7	29
Chronic humoral rejection	2	8
Chronic allograft nephropathy	3	13
Acute tubular necrosis	4	17
Pyelonephritis	1	4
Not biopsied	7	29

Fifteen of the twenty two patients underwent renal biopsy. Acute cellular rejection (29%) was the commonest histopathology followed by acute tubular necrosis.

TABLE 9:

Organism	Number	Percentage
Candida species	14	62
Aspergillus	3	13
Mucor	4	17
Cryptococcus	1	4
Pneumocystis	1	4

TYPE OF FUNGAL PATHOGEN CAUSING INFECTION

Candida species (62%) is the commonest organism causing fungal infection. The other common organisms are Mucor (17%) and Aspergillus (13%).

TABLE 10: GRAFT AND PATIENT OUTCOME OF PATIENTS WITH FUNGAL INFECTIONS

Outcome	Number	Percentage
Normal graft function	6	27
Stable graft dysfunction	7	32
Graft loss	9	41
Survived	11	50
Expired	11	50

TABLE 11: FACTORS INFLUENCING PATIENT OUTCOME

Parameter	Survived	Expired	P-Value
Deceased donor	4	1	0.000*
Living donor	7	10	
TMP	4	5	0.682
CAP	4	4	1
CMP	3	2	0.631
DGF	4	3	0.666
ART	4	2	0.362
PTDM	4	2	0.362
HBV	1	1	1
HCV	2	1	0.557
CMV	5	6	0.687
Bacterial infection	7	5	0.416
Thrombocytopenia	3	6	0.211
Leucopenia	2	9	0.001*
Anemia	6	6	1
Alb < 3.5gm/dl	3	10	0.001*
GDF	9	11	0.152
Candida	7	7	1
Pneumocystis	1	0	-
Mucor	1	3	0.291
Aspergillus	2	1	0.557
Cryptococcus	0	1	-
Graft loss	3	6	0.211
Normal graft function	4	2	0.362
Stable graft dysfunction	4	3	0.666

Student t test was used for analyzing the above data. p-value < 0.05 was considered as significant.

Fifty percent of patients with fungal infections expired. Graft loss occurred in 41% of patients. Thirty two percent of patients continued to have stable graft dysfunction.

Leucopenia and thrombocytopenia influenced patient outcome by contributing to mortality (p value - 0.001). And also more significant number of deaths occurred in patients who received renal allografts from living donor (p value - 0.001).

DISCUSSION

This study was conducted in the Department of Nephrology, Government General Hospital, chennai during the period between Aug' 08 and April' 11. Twenty two patients were diagnosed with systemic fungal infections during this period.

The mean age of the study patients was 35.55 yrs.

The male to female ratio was 1.75:1.

In a 10 year study done in Iran (from 1998 to 2008), the mean age of patients was 49 yrs and the male to female ratio was 4.2:1.[65] In a study by Chugh et al, all patients were males with a mean age of 31.05 ±7.73 years (range 21-42 years). [66]

In the present study, living donor renal transplant recipients acquired 77.5% of infections compared to deceased donor recipients (22.5%).

In the present study, Fifty five percent of fungal infections occurred with in 6 months of renal transplantation. Sixty four percent of infections occurred within a year.

According to Abbott et al, majority of the fungal infections occurred within 6 months[67]. In a study by Chugh et al, infection occurred within the first year following transplantation in seven patients and after the first year

in the others.[66] In a 10 year study done in Iran (from 1998 to 2008), 74% of invasive fungal infections occurred with in 1 yr. In another retrospective study (from 1987 to 1997) done in our department, out of 66 episodes of fungal infection 4 episodes occurred within 1 month; 28 between 1 and 6 months; and 37 after 6 months i.e., nearly 50% of the infections occurred within 6 months.[68] This phenomenon may be because of the use of numerous and higher doses of immunosuppressive agents.

In the present study, Candida species (62%) is the commonest organism causing fungal infection. The other common organisms are Mucor (17%) and Aspergillus (13%). Cryptococcus and pneumocystis constituted 4% each.

In a retrospective study done in our department, Candida was the commonest pathogen, causing 50 of the fungal infection episodes (72.5%). Aspergillus (11 episodes, 16%), Cryptococcus (3 episodes, 4.3%), Pneumocystis (3 episodes, 4.3%), and Mucormycosis (2 episodes, 2.8%) constituted the rest.[68] In the Iranian study, mucormycosis (11/21) was the commonest infection followed by Candidiasis (4/21) and Aspergillus (3/21).[65] In a study by fishman et al., candida and Aspergillus were the common organisms.[69] Infection with Cryptococcus neoformans was observed in eight patients (42%), Candida albicans in seven (37%), Mucor

species in two (11%), Aspergillus flavus in one (5.5%), and a mixed infection with Aspergillus and Cryptococcus in one patient (5.5%).

In the present study, fungal infections commonly occurred in gastrointestinal tract (GIT), lung and urinary tract, each 22%. Other sites were upper respiratory tract (15%), blood stream (11%) and central nervous system (CNS, 8%). In a retrospective study done in our department, sites of infections were GI tract (35, 50.7%), respiratory tract (18, 26%), urinary tract (8, 11.5%), CNS (3, 4.3%), and graft (2, 2.8%).The sites of infection were almost similar to that in the present study.[68]

In the literature, the risk factors for developing fungal infection in the post renal transplant setting were the following like deceased donor and retransplantation, older age, high doses of immunosuppression for anti rejection treatment, diabetes mellitus, CMV infection, bacterial infection with prolonged antimicrobial therapy, surgical interventions, indwelling catheters and anatomical abnormalities of the urinary tract.[70]

In the present study, graft dysfunction alone seemed to be a risk factor for the occurrence of fungal infection. Though many patients received anti rejection therapy (ART, 36%) and cytomegalovirus (CMV, 50%) and bacterial infections (55%), leucopenia (55%), anemia (41%) and

thrombocytopenia (41%) were present in many patients, they did not predispose to the occurrence of fungal infections. (No statistical significance).

In a retrospective study done in our department, predisposing factors were anti-rejection therapy in 24 cases, bacterial infections in 19, leukopenia in 12, tuberculosis in 7, and CMV infection in 5.[68] This difference may be due to the small number of patients in the present study. In a study by Chugh et al, graft function was normal at the time of diagnosis in 13 patients (68%) while it was impaired (serum creatinine 160umol/l) in six patients (32%). [66]

In the retrospective study done in our department, Six out of 10 diabetic recipients developed fungal infections.[68] But in the present study, only one patient had pre transplant diabetes mellitus. He developed fungal infection in the post transplant period. In a study by Chugh et al, apart from immunosuppressive drugs, predisposing factors included post-transplant diabetes mellitus in two and leukopenia in two patients. Concomitant bacterial infections were present in seven patients[66].

In the present study 50% (11/22) of patients with fungal infection died. In the retrospective study done in our department, a total of 36 out of 60 patients with fungal infection died (mortality 60%). [68]

In the present study, high percentage of deaths occurred in patients with mucormycosis (75%, 3 of 4); all of them had rhino cerebral Mucor with GDF, post transplant diabetes mellitus and leucopenia. 50% of patients with candida infections died. Three had blood stream and urinary tract infections, the remaining 4 deaths were due to candida UTI and bacterial sepsis. All those patients who had candida esophagitis survived. Thirty three percent of patients with Aspergillus (1 out of 3) and 100% of cryptococcal meningitis patients died (one patient).

In the Iranian study, 52.4% (11/21) of patients died due to fungal infection, mostly due to Mucormycosis (74%) [65].

Leucopenia (9/11, 82%) and thrombocytopenia (6/9, 66%) influenced patient outcome by contributing to mortality. And also more significant number of deaths occurred in patients who received renal allografts from living donor (10 out of 17, 59%). Their association was statistically significant.

CONCLUSIONS

1. Candida species was the commonest fungal pathogen causing infection in the renal transplant recipients (62%).
2. Gastrointestinal, lung and urinary tracts were the common sites of fungal infection (22% each).
3. Majority of fungal infections occurred in the first year (64%).
4. Graft dysfunction predisposed to the occurrence of fungal infections.
5. The mortality rate was 50%.
6. Leucopenia (82%) and thrombocytopenia (66%) were associated with high mortality.

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